CHAPTER 13, SECTION 2

MOLD ASSESSMENTS: INVESTIGATING, SAMPLING, AND INTERPRETING RESULTS

INTRODUCTION

Microbes … microbiologicals … bioaerosols … biological contaminants - all terms for the broad category of airborne particles that are living or have biological origins. This includes fungi, bacteria, viruses, protozoans, pollen, animal dander, insect parts and feces, and human skin scales. While these are all important in indoor environmental quality (IEQ) assessments, by far the most widely suspected, sampled, and publicized members of this group are the fungi.

The number of requests for mold investigations has been steadily climbing, not only in the Navy but nationwide. There are no regulations or standards for mold, so it is often difficult for occupational health professionals to interpret data. Further, most of the underlying reasons for mold contamination originate with building construction or maintenance problems, so it may be difficult for the industrial hygienist to effect the changes needed to resolve occupant complaints.

"RULES" FOR MOLD ASSESSMENTS

The following tenets are the foundation for all microbial contamination investigations:

1. **Prevention** is the best way to keep biological contamination from becoming an issue. The key element of prevention is **timely maintenance** and **prompt repair** of facilities.

2. Investigations are a **team effort**, requiring the assistance and cooperation of industrial hygiene, occupational medicine, preventive medicine, safety, occupants, labor representatives (if applicable), facilities and maintenance personnel, housing or office managers, and command public affairs officers.

3. Open, honest **communication** is vital between the personnel conducting the investigation, occupants, and management. At least one team member should be trained in risk communication.

4. If mold is found or suspected, **immediate action** is required to identify and fix the water intrusion source, dry the area, and clean or discard contaminated items. The goal is to minimize the health risk for occupants. While healthy individuals area seldom at risk from mold exposures, there is increased concern for those who are very young, old, debilitated or immunocompromised by other diseases.

INVESTIGATOR PROTECTION

1. Do not disturb contaminated areas or aerosolize biological material.

2. Do not touch visibly contaminated areas with your bare hands. If you do, wash thoroughly with soap and water as soon as possible.

3. If you have to perform destructive sampling in an area (e.g., remove a section of wallboard to access the wall cavity) or disturb a substrate that you suspect is contaminated, use appropriate personal protective equipment (PPE) and lightly spray surfaces with amended water (contains a surfactant) to minimize the possibility of aerosolizing spores.

4. Recommended PPE for those assessing and/or sampling contaminated areas includes: disposable gloves; disposable coveralls; goggles; NIOSH approved half face N-95 respirator (disposable is OK). PPE for remediation projects is discussed in the section 13.3.

COMMUNICATION

The principles and techniques learned in risk communication training are essential for mold contamination projects. Residents in Navy housing and office employees are sure to have seen some of the nationally televised programs about grossly contaminated houses and buildings with occupants who claim they can no longer function normally. There are hundreds of web sites about mold contamination, and most people have read articles about schools or homes contaminated with "toxic molds."

The more informed employees are about what is happening, the less likely they are to be fearful. Tell them in simple terms what has been found and what you will do to correct the situation. If remediation is required, tell employees what will be done, give them the remediation schedule, and explain how they may be affected (e.g., temporary relocation; control measures; testing). Provide medical support from the cognizant clinic for those with medical concerns or those who develop symptoms they believe to be associated with the contamination. Answer questions honestly and calmly, provide facts sheets tailored to the situation, and provide a contact list for medical and IH issues. Involving employees in the process gives them a stake in the successful outcome.

When mold is found, it is important to make sure that occupants are fully informed about what will be done to correct the building problems. Make sure that points of contact are identified by name so occupants can call if they have IH, medical, or remediation concerns. Section 13.5 contains a detailed discussion of risk communication.

ASSESSMENT STRATEGY

1. **Visual Inspection**. The goals of the investigation are to locate and fix the water intrusion source and to find and remove any associated contamination.

a. Always conduct a thorough visual inspection first, evaluating the building with a critical eye toward potential problem sources. Look for signs of water damage on the ceiling, walls, and floors. Inspect the ventilation system (air handling unit, ducts, fresh air intake location, dampers). Locate odor sources, and look for possible chemical and biological contaminant sources or reservoirs.

b. Likely sources or areas to check for water leaks include the roof; loose or damaged soffitts and gutters; chimneys; through-roof pipes or vents; improperly sloped drains; improperly vented appliances, uncontrolled humidity (e.g., moisture condensing on surfaces); improperly installed vapor/moisture barriers or surface finishes (e.g., exterior insulation and finish system [EIFS] or unsealed stucco).

c. Simple tests may be helpful to determine the extent of damage or contamination. For example, a boroscope can be used to check the condition of ventilation ducts. A moisture meter can quickly identify wet building materials. Assessing indoor thermal conditions (temperature and relative humidity [see ASHRAE 55-1992 or Section 13.1 for acceptable ranges] can also help identify areas where mold reservoirs are likely.

d. If mold is found, locate the source of water and repair to prevent additional water damage. Proceed with cleanup and remediation procedures in Section 13.3.

If mold is not found during the visual inspection, but the team believes there is contamination in the building (because of odors, visible water damage, employee illnesses, etc.), take additional investigative steps.

2. **Additional Investigation**

a. Review building plans and check maintenance and preventive schedules for possible relations between mechanical component locations, maintenance proceduers, and complaints.

b. Talk with employees about their complaints and symptoms, especially anything that they may have noticed different or unusual in the building or whether they detect any pattern in their symptoms or with problems in the building.

c. Check the building's relation to nearby industrial operations for potential pathways that might introduce contaminants.

d. Investigate possible hidden mold reservoirs. This may require destructive procedures, such as removing wall coverings, wall board, carpet or floor covering. Consider that there might be concealed growth behind walls, paneling or wallpaper, under floors, in electrical or plumbing chases, or in ducts.

e. Collecting screening air samples can help locate the general area of unseen mold reservoirs. Consider collecting fungi (spores), total microbial volatile organic compounds (MVOCs), mycotoxins, or glucans.

f. If mold is found, proceed with remediation per Section 13.3. Locate and fix the water source to prevent further intrusion.

If mold is not found, further investigative techniques might include investigating/testing for non-microbial causative agents. Examples include mites, allergens, or neurosensory factors (e.g., visual or perception disruptors).

SAMPLING STRATEGY

DO NOT collect samples without a sampling plan that details how and when samples will be taken, collection requirements for each type of sample, what criteria will be used to interpret results, and what benefits you expect from sampling, i.e., what question(s) will be answered and what actions will result.

ALWAYS consult the analytical laboratory before sampling to ensure sample collection and shipping are done per the lab's requirements and that results will meet your expectations.

1. When to Sample

a. **The rule of thumb in microbial investigations is Do Not Sample when visible mold is present. Regardless of the mold identified or the number of spores, it does not change the requirement to stop the water intrusion and clean up the contamination**.

This is probably one of the biggest challenges during the investigation, since sampling is a natural action for industrial hygienists and a normal expectation from occupants.

b. If you cannot collect a sufficient number of samples to fully characterize the site (i.e., because of funding constraints or insufficient sampling media), it is probably best not to collect any samples. Inadequate sample data usually lead to misleading or confusing results.

c. The investigation team should be guided by their collective expertise in deciding whether or not sampling is indicated. The following are some situations in which bioaerosol sampling is indicated:

- If an occupant has been diagnosed with a disease that is caused by a specific mold or the physician suspects an association between symptoms and mold in the workplace, the physician may request confirmation of the presence of the causative agent.

- If remediation is required, pre- and post-remediation sampling can be used to verify success of the decontamination. Surface samples are especially useful.

- If the investigation team suspects biological contamination but cannot find visible evidence, air sampling may help to verify or locate the reservoir. In

such cases, air sampling could include testing for microbiologicals (viable and/or non-viable), mycotoxins, and/or microbiological volatile organic compounds (MVOCs).

- If litigation is underway or anticipated.

- If the ventilation system was cleaned/ remediated because of microbial contamination (verified by visual or bulk/swab samples), use air sampling to determine if the areas supplied by the system are ready to reoccupy, that is, the ventilation system is not distributing bioaerosols.

d. Because you are sampling bioaerosols whose presence depends on environmental conditions (heat, light, water availability), carefully consider ambient weather conditions. For example, rain can "wash" the air clean of many spore types, such that sampling on rainy, foggy, or very humid days can result in low outdoor counts or species distributions that are significantly different from those on warm, sunny days. In general, levels of ascospores and basidiospores will be higher during rainy weather.

Sampling when there are strong winds can result in outside counts that are significantly higher than on non-windy days. In addition, high outdoor counts may mask small to moderate indoor mold problems since interpretation is dependent in part on ratios of indoor to outdoor spore counts.

Compensate for ambient conditions by adjust your sampling schedule if possible. At least be aware that outside samples may not represent normal conditions so that you do not misinterpret results.

2. **Where To Sample**

a. Complaint/problem area – Use complaint patterns, symptom descriptions, and visual indications to guide you in choosing sample locations. You may need a sampling array within a single office, on an entire floor of the building, or throughout the building to get results that are representative. Preliminary or screening samples may help target the areas that require further characterization.

b. Non-complaint area – Results serve as controls to compare with complaint area results.

c. Outside – Outside samples must be taken at the same time as indoor samples so that the types and quantities of ambient flora can be compared with those in the building. Ideally, at least one outside sample is collected at the fresh air intake that supplies the inside area being sampled.

3. **Number of Samples.** There is no formula to determine how many samples you need to adequately characterize a complaint area. Further, statistical validity considerations cannot be used because of the difficulty in predicting the environmental variability. The AIHA *Field*

Guide for the Determination of Biological Contaminants in Environmental Samples gives this guidance:

a. The number of samples depends on the size and organization of the space being investigated.

b. Sample as many locations within the area of study, control locations, and outdoors as is practically and economically feasible.

c. When possible, take duplicate side-by-side samples. According to Chapter 3 of the AIHA *Field Guide*, "duplicate side-by-side sampling is considered adequate to define the mean and the random sampling and analysis error given the high temporal and spatial variability of bioaerosol concentrations in air." …. "Acceptability of the agreement between side-by-side duplicate samples must be determined by the investigator based on the intended use of the data."

d. Investigate temporal variations by sampling at least two time periods during the day, preferably separated by a long interval, e.g., morning and late afternoon. Sample on different days or during different seasons if daily/seasonal variations appear to influence conditions.

4. **Choosing the Appropriate Type of Sample – Bulk, Surface, Air**

Before taking a sample, think about why you need the result and what you want the results to tell you. If you're trying to determine if an area is contaminated or if what you see is really mold, a swab or bulk sample is sufficient. If you are trying to support a medical diagnosis, a viable sample is needed for the lab to identify and speciate the mold.

A brief discussion of the methods to collect fungal samples for identification and/or quantification follows. Appendix 13.2-A summarizes sampling methods, their strengths and limitations, and provides resources for more information.

a. Bulk samples – Used to identify contaminants, especially when trying to locate or confirm the presence of a mold species as a causative agent for medical diagnosis. Examples of materials that might be collected include carpet, insulation, duct lining, wallpaper, or wallboard (sheetrock).

Collect samples from visibly contaminated surfaces by scraping or cutting with clean tools (e.g., wall board). Place sample in a clean, plastic bag and label for transport.

Bulk water samples can be collected from condensate drain pans, cooling towers (i.e., for Legionella), or other water reservoirs suspected of being a contaminant source. Collect in a sterile container, seal tightly, and transport in a secondary container such as a ziplock bag to contain the sample in case of breakage or leaks.

Another type of bulk sample can be taken using a microvacuum. This is basically a cassette attached to a pump that is used to vacuum carpets, furniture, or other substrates to collect the

particulate matter. Though the sample can be randomly vacuumed into the cassette, using a specific grid collection area will allow quantitative results.

b. Surface samples – Surfaces can be sampled by swabbing or using clear cellophane tape (also called a "sticky tape" sample). The sample is analyzed by direct microscopic examination to determine if there is microbial contamination. Sterile swab collections can be cultured for identification. Surface sampling is limited to identifying settled fungi or spores and may not be related to airborne results.

c. Settling plates/ gravity plates – Open nutrient agar petri dishes are placed on a flat surface to collect anything that settles out of the air. Results are not particularly meaningful, since what grows depends on random settling of airborne particulates onto a non-specific growth medium. Navy personnel will not use this method.

d. Air samples. Air sampling is the most common collection method for bioaerosols. A pump is used to draw in air and deposit the particulate onto a collection medium. Most air sampling methods can be used for microscopic analysis, a few for culturing techniques, and others for specialized testing. Each kind of air sample has its benefits and disadvantages, depending on the media used and the collection and analytical method chosen. Regardless, air sample results for molds are subject to false negative results. That is, there may be contamination present even when results indicate otherwise. Consider:

(1) Most samples are very short, and therefore capture only 5 - 10 minute snapshots of what is actually happening at the sample location.

(2) You have to decide whether you want viable or non-viable results (culturable or non-culturable, respectively) before you sample.

(3) If the sampling method does not have sufficient collection efficiency in the size range(s) of the mold present, you will not collect the spores.

(4) If collecting samples directly onto agar (viable sample), you are likely to miss molds that have special growth requirements.

5. **Choosing the Sampling Method**. Appendix 13.2-A summarizes sampling methods, advantages/ disadvantages, and provides information resources. You may want to refer to the chart during discussion.

a. Viable (culturable) vs. Non-viable (non-culturable) samples

(1) Viable (culturable) samples are collected on nutrient agar initially, or can be collected in/on inert media and prepared for culture at the laboratory. Samples are incubated for several days to allow cell growth and replication into visible colonies. The entire colony, not just the spore, is used for the identification, allowing the lab to make a more exacting identification of certain mold types.

Culturable samples tend to underestimate the number of total spores present, since only viable organisms will grow. Of the viable fungi that impact onto the agar during sampling, only a percentage of those will actually grow during incubation.

Further, remember that some molds require specific nutrients or growing conditions. If these are not present, the organism will grow very slowly or not at all. For example, *Stachybotrys* requires cellulose. If you use Malt Extract Agar (MEA) for sampling and the report shows no *Stachybotrys*, this means that (1) there really was no *Stachybotrys* in the sampled area; or (2) *Stachybotrys* was present but MEA did not support its growth. If you suspect *Stachybotrys*, contact the laboratory to determine the nutrient agar of choice (usually Rose Bengal or cellulose agar) for collecting viable samples. A better alternative is to collect a non-viable sample since the spores are very distinctive and can easily be identified by direct microscopic examination.

You can also get relatively good recovery of fungi (about 60%) if you collect the sample using a button sampler and filter media. Note that if you use this method to collect a bacteria sample, recovery is <20% due to cell dessication. You can increase bacteria recovery to around 60% by using a gelatin filter.

(2) Non-viable samples are examined directly under a microscope to identify and count spores and other particulates (e.g., fibers, skin cells, mycelial fragments) based on morphological features. Some molds, such as *Aspergillus* and *Penicillium*, cannot be distinguished by their spores alone, so they are reported as a group, e.g., *Aspergillus/ Penicillium* group; *Drechslera/Bipolaris* group; or Smuts/*Periconia*/*Myxomycetes*.

b. Bioaerosol collection principles. In general, collecting bioaerosols involves either filtration or impaction. Figure 13.2-1 shows the collection methods and the possible analyses that can be performed using each.

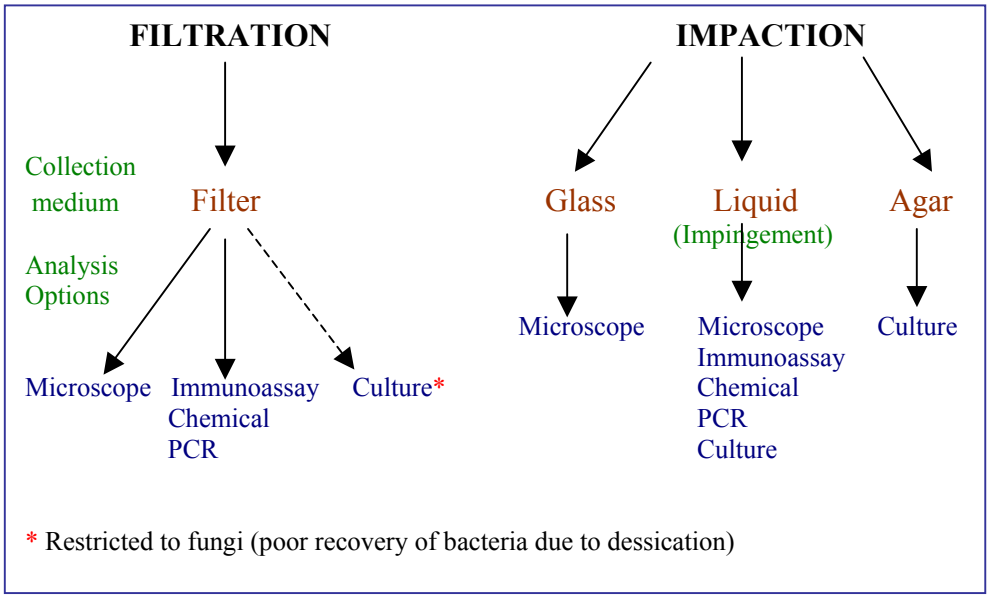

Figure 13.2-1. Bioaerosol collection methods.

(1) Filtration - Bioaerosol is collected on a filter as air passes through it. Filter media can have different diameters, pore sizes, and composition, so consult the laboratory before sampling.

(2) Impaction – Bioaerosol is impacted onto a collection media such as glass (may contain collection strip of agar, grease, adhesive, or tape), agar plates, or liquid. Impaction into a liquid medium is also called impingement.

c. Why mold size is important

Particle collection efficiency is driven by the size of the particle you want to collect. With spores, this can make the difference in whether a negative result means there really is no mold present – or that the mold is there but you didn't collect it.

Appendix 13.2-A lists collection efficiencies of some of the more commonly used methods. For example, if you suspect that you have *Cladosporium cladosporoides* contamination and you sample using a Burkard spore trap (impaction onto slide), you'll probably get negative results. Notice that the 50% cut size of the Burkard sampler is around 2.5 µm. Since *C. cladosporoides* is around 2 µm, you will miss most – if not all – of the spores simply because the collection device is inefficient (about 10%) at 2 µm.

6. **Sampling Tips**

a. Before collecting any samples, select the analytical lab you will use. Call the lab to ensure that you sample according to their requirements, especially if you are taking viable culture samples.

b. In some cases, the laboratory may provide the sample collection equipment. For example, most labs will loan you an Andersen N6 and provide the correct agar for the targeted biological population. For non-viable sampling, you will have to purchase the sampling media (e.g., Air-O-Cell cassettes), but the lab may loan you the high volume pump.

c. Sampling conditions should be reflective of "normal" building conditions. The ventilation system should be on the usual daily setting (i.e., temperature, damper opening(s), setbacks, auxiliary/booster fan operation, fresh air intake settings, etc.) and employees should work as they typically do. DO NOT intentionally alter the area to be sampled.

d. Sample on different days and at different times of the day to get samples that represent conditions over time. Replicate samples are a good idea to increase confidence in your results. Remember that results tend to be less reliable or repeatable when sample times are very short!

e. Aggressive sampling is not recommend for investigational studies. While aggressive techniques will disturb accessible mold reservoirs, it complicates result interpretation because it is not representative of normal building conditions.

f. Record ambient conditions during sample collection, such as temperature and relative humidity. Also make notations of conditions inside that may impact results, such as obvious water damage or contamination in relation to the sample location; potential microbial reservoirs, like fish tanks, plants or trash; condition of HVAC system components; presence of pets; or open/ leaky doors and windows. Outside sample notes should include weather conditions (cloud cover, recent precipitation, wind) and locations of land features (ditches or standing water, landfills, playgrounds, construction areas).

g. Chain of Custody (COC). It is prudent to use a COC form with your bioaerosol samples. The COC is particularly important should you become involved in litigation, but should be used anyway to track the samples' journey from collection to analysis. If you don't have a COC form, most labs will supply you with one. You can view examples at the following sites:

http://www.aerobiology.net/COC.pdf
http://www.aerotechlabs.com/InfoBase/cocs.aspx
http://www.emlab.com/media/resources/submit.pdf
http://www.emsl.com/new_chain.pdf
http://www.stl-inc.com (Go to Our Labs > P&K Microbiological > Chain of Custody)

7. **Sampling for Fungal Metabolites and Cell Components**

a. Microbial Volatile Organic Compounds (MVOCs)

(1) MVOCs are produced by molds that are metabolically active. They are also responsible for many of the musty odors associated with molds. If you 'smell mold' but cannot see it, MVOC sampling may help to locate the fungal reservoir.

(2) Collect samples using low flow pumps and sorbent tubes as specified by the lab. Ship samples on ice and protect from heat and light.

b. Mycotoxins

(1) Fungi are primarily saprophytic, that is, they use nonliving organic material as the nutrient source for growth and reproduction. During the digestion process, fungi secrete enzymes to help break down complex compounds into simpler ones that can be taken up and digested. The by-products of digestion are classified as primary or secondary metabolites.

(2) Primary metabolites are produced from cellulose and other compounds that are used by the fungus for energy, growth, and reproduction. Secondary metabolites, called mycotoxins, are natural by-products that are not necessary for growth and are usually derived from precursors formed during primary metabolism. They are thought to give the fungi a competitive edge against other microorganisms, including other fungi.

Whether a toxigenic fungus actually produces mycotoxins appears to depend on environmental conditions, including temperature, growth substrate, and pH. Some of the mycotoxins most

commonly associated with mold contamination in buildings are briefly described in Appendix 13.2-B.

(3) Exposure. Mycotoxins accumulate in spores, mycelium, and growth substrates. Consequently, they can be inhaled (when spores or substrates are disturbed and aerosolized), ingested (consuming toxin-containing spores when eating, drinking or smoking in a contaminated area), or absorbed through the skin (e.g., when handling contaminated materials).

(4) Health Effects. Symptoms associated with exposure to mycotoxins include dermatitis, cold and flu symptoms, sore throat, headache, fatigue, diarrhea, inflammatory reactions, and impaired or altered immune function (which can lead to opportunistic infection). Many toxin-producing fungi, such as *Stachybotrys, Penicillium, Aspergillus* and *Fusarium* species, have been linked to illnesses resulting from exposure to fungi growing in water-damaged buildings. Other mycotoxins have been associated with cancer (e.g., aflatoxins from *Aspergillus*), cardiovascular effects (ergot alkaloids), and neurological symptoms (*Aspergillus fumigatus*).

(5) Sampling and Analysis. Contact the laboratory before collection for specific lab instructions. Because air sampling for mycotoxins has limitations, bulk, surface, or dust samples are usually best.

- Air - Collect on filter cassette. Store samples at ambient temperature under desiccated conditions.
- Dust/Bulk - Select an area with visible contamination, and collect 25-50 grams of material in a suitable container. Microvaccuming and surface swab (methanol swab) techniques can also be used.
- Water - Collect 5 ml of water. Seal. Refrigerate and ship to the laboratory via overnight courier under refrigerated conditions.

c. Glucans and Ergosterol

(1) Both (1→3)-β-D-glucan (glucans) and ergosterol are fungal cell wall components of filamentous fungi, which includes most saprophytes. These compounds have been sampled successfully as chemical markers to show that such fungi are present. Sampling will detect ergosterol in both living and dead spores (ergosterol is fairly stable in spores).

(2) Collect glucans on a membrane filter, extract, and analyze using a Limulus amoebocyte lysate (LAL).

(3) Ergosterol sampling is also done on a filter that is extracted to remove the ergosterol. Analysis can be done using high performance liquid chromatography (HPLC), gas chromatography (GC), or GC with mass spectrophotometry (MS).

(4) There is little data comparing the number or mass of spores to chemical marker concentrations.

d. PCR (polymerase chain reaction) analysis provides genetic confirmation of certain fungal species using species-specific DNA probes or primers. PCR is quick and specific, but the technology is limited to the species probes available for fungal confirmation.

Consult the individual laboratories that offer PCR to determine what fungi are in their detection panels. Some of the available probes/primers of interest developed to date include: *Alternaria alternata; Aspergillus flavus, fumigatus, niger, sydowii, versicolor; Chaetomium globosum; Cladosporium cladosporoides; Penicillium aurantiogriseum, brevicompactum, chrysogenum expansu, griseofulvum, purpurogenum, viridicatum; Stachybotrys chartarum; and Ulocladium botrytis.*

8. **Sampling for Bacterial Cell Components: Endotoxins**

a. Endotoxins are found in the cell walls of gram negative bacteria. Made of lipopolysaccharides, they can elicit health effects in susceptible individuals whether the bacteria is viable or not. The most common exposure routes are inhalation and ingestion. Gram negative bacteria are most often associated with water, sewage, humidifiers, and gray/black water contamination.

b. Air samples are collected using endotoxins-free polystyrene cassettes. The samples must be collected carefully to ensure there is no human contamination.

Bulk water samples can be taken in endo-free vials, again using sterile techniques, and must be kept on ice for shipment to the lab.

SAMPLE ANALYSIS

1. Use only analytical laboratories that are proficient in the Environmental Microbiology Proficiency Analytical Testing (EMPAT) program. The EMPAT evaluates the lab's ability to correctly identify cultured fungi and bacteria that might be found in mold contamination investigations. Under the current program, labs must correctly identify the genus, and they receive bonus points for correctly speciating the organism. The EMPAT certificate states whether the proficiency is for identification of bacteria, fungi, or both.

At this time, proficiency testing does not involve counting (of spores or colonies) or identifying organisms from mixed cultures.

Beware of laboratories that advertise that they *participate* in the EMPAT rather than that they are *proficient* in the EMPAT.

The American Industrial Hygiene Association (AIHA) administers the EMPAT. Consult their web site at http://www.aiha.org/LaboratoryServices/html/micro.htm for the most current proficiency testing results and accreditation category.

2. While not required at this time, it is recommended that analytical laboratories also be accredited through the Environmental Microbiology Laboratory Accreditation Program (EMLAP). This program assesses and rates various lab parameters, such as: personnel qualifications, EMPAT scores (performance), facilities, quality assurance programs, record-keeping, analytical methods, and operating procedures. EMLAP also includes triennial site visits to the laboratory. Details are at http://www.aiha.org/LaboratoryServices/html/micro.htm.

3. Consult with the laboratory to define sample collections methods, turnaround time, costs, shipping requirements, and exactly what the analysis report includes. For example, some labs clearly report genus and spore count, e.g., *Cladosporium* 450 spores/m^3, while others may report results as *Cladosporium*-like (not definitive for *Cladosporium* but spores look similar) or *Cladosporium* 42 spores (you have to calculate concentration).

4. Appendix 13.2-C provides a consolidated list of environmental microbiology laboratories, bioaerosol services available, accreditation status, and contact information. Please provide changes or additions to IH-Director@nehc.mar.med.navy.mil.

INTERPRETING RESULTS

The presence of mold does not mean that occupants will have adverse health effects or that they will even be exposed. Like any other stressor, you must have a completed exposure pathway to the biocontaminant. The mold or mold fragments, spores, or metabolites must be produced, released, reach the occupants, then be inhaled, physically contacted, or ingested. Even after contact, human response will depend on individual susceptibility (e.g., genetic predispositions to allergens, age, health status) and type of exposure (allergen, toxin, infectious agent).

There are no standards for biological sample results. The American Industrial Hygiene Association, American Conference of Governmental Industrial Hygienists, Environmental Protection Agency, and numerous other resources agree that the best criteria for interpreting results is to compare inside samples with outside and/or contaminated areas with uncontaminated areas, along with consideration of both the kinds of mold present (genus/species) and the numbers (spore or colony counts).

Interpretation Criteria

a. Compare Indoor and Outdoor Results. An effective interpretation is based on comparing inside and outside sample results. In general, inside counts should be around 30-80% of outside and have the same general distribution of genera.

- Rank order the genera/species results. The relative order inside should be similar to outside. If the dominant types of mold in indoor samples are not the same as those in

outdoor samples, it indicates an indoor mold source.

- The concentration of each genus/species identified inside should be less than outside. Higher inside levels indicate there is fungal amplification indoors.

- The presence or absence of a few genera in small numbers should not be considered abnormal.

- Normal outside fungi typically include *Cladosporium*, *Alternaria*, *Epicoccum*, and Basidiomycetes, so it is common to see these identified in indoor samples.

- The presence of certain fungi indoors should prompt immediate risk management decisions. Examples of fungi of concern include *Aspergillus versicolor, Aspergillus flavus, Aspergillus fumigatus, Stachybotrys chartarum*, *Fusarium moniliforme*, *Histoplasma, and Cryptococcus*.

- Numerical guidelines can be useful as a secondary interpretive resource when evaluating viable sample results (i.e., reported in colony forming units per cubic meter of air (CFU/m^3)). Fungi levels in excess of these numbers do not mean that the conditions are unsafe or hazardous. Do not use these guidelines for non-viable sampling results.

  < 150 CFU/m^3 total fungi is acceptable if the reported genera are reflective of normal outdoor flora (e.g., *Cladosporium* and other leaf and tree fungi).

  < 500 CFU/ m^3 total fungi is acceptable in summer if the reported genera are reflective of normal outdoor flora.

  > 50 CFU/m^3 of a single species other than *Cladosporium* or *Alternaria* should prompt further investigation.

  >1000 CFU/m^3 total fungi indicates potential building related problems and requires further investigation.

b. Consider Outside Air Entry.

- Filtered or conditioned air will affect the relative numbers of genera. In an office building with little fresh outside air or poor air exchange rates, 'normal' inside counts may be very low, i.e., 2-5% of outside. The rank order of genera should be similar.

- If sampling in a building or residence when doors and/or windows are open, expect 'normal' inside counts to be very similar to outside – as high as 95%. The rank order of genera should be similar.

c. Put Results in Context With Other Facts.

- On microscopic examination, morphologically similar spores cannot be differentiated. The most common example of this is with *Aspergillus, Penicillium, Gliocladium, Trichoderma*, and other small, round, colorless spores. Non-culture results will report all such spores as *Aspergillus/Penicillium* group.

  If results show high indoor counts of *Aspergillus/Penicillium*, you may want to collect samples for culture to separate the genera and determine which species of *Aspergillus* is present, since several produce mycotoxins and are infectious.

- Myxomycetes/Rust/Smut or Smuts/Periconia/Myxomycetes will also be reported together on non-cultured sample reports. These are morphologically similar round, brown spores that are common outdoor plant molds.

- The presence of fungal fragments such as hyphae or conidiophores suggests colonization, growth, or accumulation of fungi in the sampling location.

- The presence of yeast suggests wet conditions.

- Be sure you know the ambient sampling conditions before using outside results:

  Outside samples collected during or soon after rain will usually have lower total spore counts but higher relative concentrations of ascospores and basidiospores.

  Expect higher concentrations of fungi in warmer weather, lower total counts in cooler weather.